DETERMINATION OF PROTEIN AND SUGAR CONCENTRATION IN MAIZE SUBJECTED TO CLINO-ROTATION

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SEPTEMBER, 2023.

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A PROJECT SUBMITTEED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE. IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY.

(BIOTECHNOLOGY TECHNIQUES)

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CERTIFICATION

This is to approve that this project work was carried out by Simon Urukpose UGHE with matriculation number LSC1810354, under the supervision of DR. ALEXANDER ORUKPE of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, Benin City.

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DECLARATION

I declare that the project work; The determination of protein and sugar concentration in maize crop subjected to clino-rotation was written by me in the Department of Science Laboratory Technology (Biotechnology Techniques), University of Benin, Benin city, Edo state.

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Simon urukpose UGHE DATE

DEDICATION

This project is dedicated to God Almighty for his love, grace, strength, provision, and protection throughout my final year research and laboratory work. Secondly to my parents Mr. and Mrs. Lucky Ughe, and my siblings for their undiminished support and massive assistance throughout the whole exercise.

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I am indeed grateful to Almighty God for his wisdom and provision throughout the period of my academic journey in the University of Benin.

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ABSTRACT

The search for extraterrestrial life has been of major concern to scientists as they try to seek for ways by which humans and other living organisms can survive in outer space and beyond. Therefore, studying the effects the space environment could have on living systems and, most likely, finding solutions to these problems has been of great concern to scientists. Microgravity, or the condition of almost zero-gravity, can have profound effects on living systems, including plants and humans. The study design involves subjecting maize seeds to clinorotation condition within a controlled laboratory setup. The experiment was conducted in several replicates, with conventional conditions serving as the control group. The clinostat stimulated micro gravity condition, facilitated continuous rotation of the maize seed, removing the effect of gravitational force. The effects of microgravity on plants include changes in growth and morphology, changes in nutrient uptake, changes in response to light and changes in gene expression, while the effects on humans include changes in bone density, muscle atrophy, fluid shifts, cardiovascular changes and immune systems changes. Several plants can be used for space explorations. Maize plant can survive in a microgravity environment and can serve as a food source (providing good protein and sugar content), and provide oxygen which can be used by astronauts for long space exploration. The effect of microgravity can could impair the growth and development of the plant, but the use of stimulators like IAA (indole-3-acetic acid), SNP (sodium nitroprusside) will significantly reduce the effects of microgravity environment on *zea mays* during long space exploration. Understanding these effects is important for developing effective countermeasures to mitigate their negative effects and ensure the safety and health of astronauts in space.

CHAPTER ONE

INTRODUCTION

Living systems are a fundamental aspect of the natural world, encompassing all living organisms and their interactions with each other and their environment. They are characterized by their ability to respond to changes in their surroundings, maintain homeostasis, and evolve over time. Living systems are studied in various fields such as biology, ecology, and systems science. One of the key properties of living systems is their ability to maintain homeostasis, which is the process of maintaining a stable internal environment. Homeostasis involves a range of mechanisms that help to regulate temperature, pH, and nutrient levels, among other things. For example, when the body temperature rises, humans and other animals will begin to sweat to cool themselves down. Plants also have mechanisms to regulate their internal environment, such as opening or closing their stomata to conserve water in response to changes in humidity.

Plants are multicellular organisms that belong to the kingdom Plantae. They are characterized by their ability to use sunlight, carbon dioxide, and water to synthesize their own food through the process of photosynthesis. Plants can be found in a wide range of environments, from deserts and forests to oceans and wetlands. They are an important part of many ecosystems, providing food, shelter, and oxygen to other organisms. Plants come in a variety of sizes, shapes, and colors. Some are large trees that can live for hundreds of years, while others are small herbs that only live for a few months. Plants also have a wide range of adaptations that allow them to survive in different environments. Plants are not only important for their role in ecosystems, but they also have many practical uses for humans. They are a source of food, medicine, and materials such as wood and fibers. Growing plants in space requires a deep understanding of plant growth mechanisms and proficient know-how in controlled-environment agriculture (Kiss *et al.,*2014). For many decades, healthy plants have grown on spacecraft environments; (Kiss *et al.,*2014), gave an extensive review of the evolution of plant growth experiments in space and associate hardware in the years 1960–2000. However, long-term effects of the space environment on plant growth and reproduction are not yet well known and understood, and could impact the role of plants as food source in bio-regenerative life-support systems (Wolff *et al*.,2014). Plants will play a critical role in the survival of human beings outside Earth for long-durational missions within the Solar System.

Space is the region immediately outside of the Earth's atmosphere, it can also be referred to as “OUTER SPACE”. Our earth’s atmosphere is divided from the outer space by an imaginary line called “The Kármán Line” it is where space officially begins. It starts for Earth at a height of about 100 kilometers (62 miles). At this point there is no considerable air to breathe or light to scatter, because there is no enough oxygen (O_2) molecules in the air above to keep the sky blue. The search for extraterrestrial life has been of a major concern to scientists as they try to seek for ways by which humans, plants and other organisms can survive in the outer space and beyond.

Microgravity is the condition whereby people or objects appear to be weightless and experience no force of gravity. It is sometimes referred to as “zero gravity” but zero means nothing which is literally wrong as micro means “very small” meaning there is very little amount of gravity present (NASA, 2017).

Gravity refers to the force that attracts two objects towards each other, The strength of this force depends on the mass of the objects and the distance between them. The Earth's gravity creates a gravitational environment that affects all objects on or near its surface. This environment is what allows us to stand on the ground and keeps the moon and satellites in orbit around the Earth.

The concept of gravity was first introduced by Sir Isaac Newton in his laws of motion and later confirmed by Einstein's theory of general relativity.

Aim

The purpose of this study is to determine the protein and sugar content on maize plant (*Zea mays*) subjected to a clinostat and grown in the Space Earth Environment Research Laboratory, at the University of Benin, Edo state, Nigeria.

Objectives

The following objectives were carried out in this research work

1. Determination of the total sugar contained in the maize plant both grown under micro-gravity and controlled environment, conducted in the Space Earth Environment Research Laboratory in University of Benin, Edo state, Nigeria.
2. Determination of protein of the *Zea mays* grown under a stimulated micro-gravity environment using a clinostat and a controlled environment (gravity).
3. Determination of the yield parameters of the *Zea mays* grown under micro-gravity and a controlled environment.

CHAPTER TWO

LITERATURE REVIEW

According to Soga *et al.,* (2002), humans who go on board on a spacecraft will need metabolic energies for their bodies in addition to the energy required to power the spacecraft. Traveling through space could take years, and since humans are heterotrophs, resources such as oxygen for metabolic energy production would be needed in the form of food. Furthermore, these two forms of human sustenance-based criteria for space travel are mostly plant-based. Plants, for example, have long been thought to be the best oxygen generators, considering the use of synthetic methods (Stutte *et al.,* 2002). Plants consume CO_2 which is a by-product of human metabolism, and then give off oxygen, which is needed for human survival. Photosynthesis is also very important, especially in driving the process for generating ATP, which humans require for metabolism (Papaseit *et al.,* 2000). Secondly, the primary producers in food chains are plants (Kering and Zheng, 2015), so much so that plants cannot be taken away from the human mechanism of survival. During space travel, one of the greatest effects on plants is the influence of gravity (Vandenbrink *et al.,* 2014; Kiss *et al.,* 2019; Orukpe *et al.,* 2021). This ubiquitous force usually influences plant development, productivity, and morphology at all levels, from the molecular level to the whole plant (Vandenbrink *et al.,* 2014). Previous studies have shown that changes in gravitational forces usually affect growth and development in plants. plants will be crucial in ensuring resources for human existence on long-duration journeys beyond Earth during space colonization. Microgravity conditions in space offer an unparalleled environment to study reduced gravity's effects on animal and plant organisms. Plants in space may not only supply future food, but also oxygen for survival. Plant experiments have been performed in space since the launch of Soviet/Russian Salyut 1, the first space station. Plants have also been cultivated in various space-mimicking environments for a long time now. As a food source in bio-regenerative life support systems, plants may be affected by the long-term impacts of the space climate (Wolff *et al,.*2014). To cultivate plants successfully in space, we must first deeply understand plant growth mechanisms and behaviour in microgravity situations (Kiss *et al.,*2019). Gravity regulates buoyancy, convection, and sedimentation, which all influence a range of physical and chemical processes also associated with plant growth and development. The ubiquitous force of gravity impacts plant growth, development, and morphology at all levels. To study this, plants have been subjected to microgravity in laboratory on Earth. Here on Earth, short-duration microgravity conditions are simulated by approaches such as rotation in clinostat. It has been shown that a gaseous environment was very important for plant growth and development in space. It was initially thought that microgravity led to smaller plants and that their reproduction was impaired (Musgrave *et al.,* 2005; Paul *et al.,* 2013) but as plant growth hardware for space improved, these artefacts disappeared and it was shown that plants can grow normally in microgravity provided that the plants grow in a well-ventilated area (Monje, Stutte and Chapman 2005). A major result in space biology was the demonstration of a seed-to-seed growth of *Arabidopsis thaliana* on orbit during STS-68 mission, which showed no difference with ground controls (Kuang, Xiao and Musgrave 1996; Paul *et al.,*2013).

2.1 Microgravity Environment

Microgravity refers to a state or condition whereby people or objects appear to be weightless and experience no force of gravity. It is sometimes referred to as "zero-gravity" but zero means nothing which is literally wrong as micro means "very small or minute" meaning there is very little amount of gravity present (NASA, 2017).

The whole concept of microgravity was first explored during the early days of human spaceflight. The first human to experience microgravity was astronaut Yuri Gagarin, who orbited the Earth on April 12, 1961. Since then, microgravity has been a central of human space exploration and has provided researchers with a unique environment for studying the effects of gravity on various physical and biological processes.

Microgravity environment can be found in outer space, some certain type of aircraft and in some laboratories on earth (stimulated micro-gravity). The most notable characteristic of microgravity is the absence of a gravitational force pulling objects towards the center of the Earth, this means that objects in microgravity will float freely and move in any direction without any resistance, this type of environment can be challenging for plants, as is not adapted to the lack of a gravitational force.

One of the main benefits of microgravity is its ability to provide an environment where scientists can study the effects of microgravity on various physical and biological processes. In microgravity, fluids and particles behave differently than they do on Earth, and this allows researchers to study N a wide range of phenomena, including combustion, fluid dynamics, and materials science. For example, microgravity has been used to study the behavior and effect of plant growth and development in space, which can help scientists better understand the effect of micro-gravity on these plants. An equipment known as a clinostat is used to simulate microgravity on earth in order for scientists to be able to study microgravity and its effects on plants.

Figure 1: A clinostat used to stimulate a micro-gravity environment

(Orukpe *et al.,*2021)

The effect of microgravity on plants has been a subject of interest for researchers and scientists for many years. Microgravity refers to almost complete absence of gravity experienced by objects in orbit around the Earth, such as spacecraft or the International Space Station (ISS). Some of the effects of microgravity on plants include;

1. **Changes in growth and morphology**: In the absence of gravity, plant roots no longer grow towards the ground, and shoot growth may also be affected. Plants grown in microgravity may have a different morphology compared to those grown on Earth. For example, they may appear more branched and bushy (Ferl and Paul, 2016; Kwon *et al*., 2019).

2. **Changes in gene expression**: Plants grown in microgravity may show altered gene expression patterns, which can affect their physiology and metabolism. For example, genes involved in stress responses and cell wall modification may be upregulated in microgravity (Correll *et al*., 2013).

3. **Changes in nutrient uptake**: Plants grown in microgravity may have difficulty taking up nutrients, as they no longer experience the force of gravity which helps to pull water and nutrients towards their roots (Correll *et al*., 2013).

4. **Changes in response to light: In microgravity**: The directional cues provided by gravity are absent, and this can affect how plants respond to light. For example, the leaves of some plants grown in microgravity may orient themselves perpendicular to the direction of light, rather than parallel as they would on Earth (Stutte *et al.*, 2002; Paul *et al*., 2012).

2.2 EFFECT OF MICROGRAVITY ON LIVING SYSTEMS

Microgravity, or the condition of almost zero-gravity, can have profound effects on living systems, including humans, animals, plants, and microorganisms. During space travel, one of the greatest effects on plants is the influence of gravity (Levine, 2010; Vandenbrink *et al*., 2014; Braun *et al*., 2018; Kiss *et al*., 2019; Orukpe *et al*., 2021). This ubiquitous force usually influences plant development, productivity, and morphology at all levels, from the molecular level to the whole plant (Vandenbrink *et al*., 2014). Previous studies have shown that changes in gravitational forces usually affect growth and development in plants as well as changes in the human body. Below are some of the effect of micro-gravity on humans. They include:

1. **Changes in bone density**: In microgravity, there is no longer the force of gravity acting on the bones, leading to bone loss and decreased bone density. This can result in a higher risk of fractures and other bone-related problems (Miller and spoolman, 2020). The **COLBERT machine** is a treadmill Famously named after comedian Stephen Colbert, the new running machine helps astronauts stay fit, fighting off the bone loss and muscle decay that otherwise comes with space travel. The bungee cords are used by the astronaut to strap themselves to the treadmill as it helps to keep them grounded while running.

2. **Muscle atrophy**: Without the constant resistance provided by gravity, muscles in the body can weaken and atrophy. This can result in reduced strength and endurance. (Massa *et al*., 2013)

3. **Fluid shifts:** In microgravity, fluids in the body tend to shift towards the upper body, causing fluid accumulation in the head and neck. This can lead to a puffy face, nasal congestion, and decreased visual acuity. (LeBlanc *et al*., 2000; Massa *et al*., 2013)

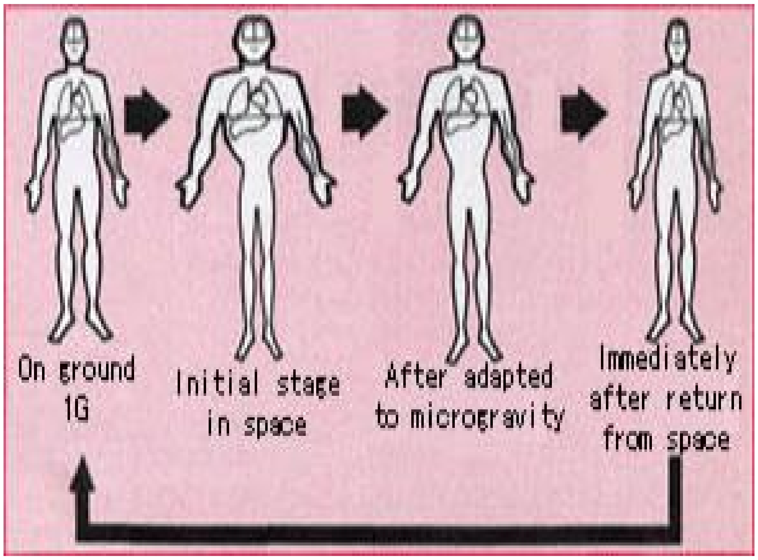


Figure 2: Representation of fluid shift occurring on astronaut in gravity and micro-gravity.

(Crucian *et al.,* 2018)

4. **Cardiovascular changes**: The cardiovascular system can also be affected by microgravity, as blood no longer needs to be pumped against the force of gravity. This can result in a decrease in overall blood volume, changes in blood pressure, and altered heart function. (Kiss *et al*., 2019; Hughson *et al*., 2016)

5. **Immune system changes**: The immune system can be affected by microgravity, leading to a decreased ability to fight infections and a higher risk of developing certain illnesses. (Crucian *et al*., 2018)

6. **Psychological effects**: Living and working in a confined space with other people for long periods of time can also have psychological effects, such as mood changes, stress, and anxiety (Hughson *et al.,* 2016)

7. **Nervous system**: In microgravity, changes in sensory input can lead to spatial disorientation, motion sickness, and altered perception of the body. (Kornilova *et al*., 2003;   Hughson *et al.,* 2016).

2.3 Effect of Microgravity on plant growth and development

In early spaceflight experiments on plant growth and development, many observed effects that were attributed to microgravity were actually due to indirect spaceflight effects (Paul *et al.,* 2013), such as confinement, leading to build-up of volatile organic compounds, such as ethylene and lack of convection resulting in elevated CO_2 levels in the spacecraft and at plant-leaf surfaces (Monje *et al.,*2003). Elevated radiation levels also have negative effects on plant

growth and development. On-orbit plant experiments can therefore be confounded by these spacecraft-specific artefacts (Musgrave 2002).

The effects of microgravity on plant growth and development have been thoroughly reviewed in recent years (Wolverton and Kiss 2009; De Micco *et al.,* 2014; Paradiso *et al.,*2014; Wolff *et al.,*2014; Kittang Jost, Hoson and Iversen 2015; Vandenbrink and Kiss 2016) . Radiation and magnetic field are also known to affect plant growth. As reviewed in Arena *et al*., (2014). plants can sustain radiation doses one hundred times higher than mammals can and low radiation doses might lead to positive outcomes such as increase in growth and photosynthesis. Their effects are thoroughly reviewed in De Micco *et al.,* (2011) and Wolff *et al.,*(2014). it was shown that, with adequate ventilation in space, plant development is similar to that on Earth (Monje, Stutte and Chapman 2005), secondary metabolism is affected by altered hypo- and hyper-gravity as reviewed by Tuominen, Levine and Musgrave (2009) and major changes in storage reserves were observed in the spaceflight environment, with seeds produced on orbit having different composition and developmental stages than seeds grown on Earth (Musgrave *et al.,*2005), but also on clinostats (Brown, Piastuch and Knott 1994). These changes in starch storage and metabolite production could impact the vigour and nutritional content of seeds produced in space plants (Musgrave, Kuang and Matthews 1997) and might affect the flavour of plants produced in space (Musgrave *et al.,*2005), which could become a problem on long-duration space missions where crews would rely on plant-based diets. Cell growth and proliferation are two related processes that are coupled in gravity but they seem to be decoupled in microgravity, which could have an impact on plant growth and development and hence on food production in reduced gravity environments (Medina *et al.*,2015). Manzano *et al*., (2009) found that cell proliferation was enhanced, whereas cell growth was decreased in microgravity; they hypothesized that this decoupling was due to acceleration in cell division caused by a shorter G2 phase, leading to the formation of more cells with shorter sizes (Manzano *et al.,*2009). This result was confirmed in 2010 by Medina *et al*., who found that meristematic cell proliferation was enhanced while meristematic cell growth was reduced in microgravity (Medina *et al.,*2010). This was reviewed by Herranz and Medina in 2014, who hypothesized that changes in cell growth and proliferation in non-specialized cells in microgravity could be a result of gravity resistance mechanisms (Herranz and Medina 2014). Many flight experiments demonstrated that plants develop normally in microgravity (Ferl *et al.,*2002), i.e. the overall plant build up is not altered by the lack of gravity, as long as adequate ventilation is provided. However, the pattern of root growth is more random than what is observed in ground controls, especially lateral roots. they tend to develop much more in microgravity instead of having a large primary root (Ferl *et al.* 2002).

Plants tropisms have been extensively studied. For example, it was shown that *Arabidopsis thaliana* seedlings had a greater phototropic response to blue light in microgravity than in Earth gravity (Millar *et al.*,2010). Studies of plant roots in weightlessness have revealed much about the metabolic pathways of gravitropism and how plants grow according to the direction of the gravity vector, as well as about the mechanism of gravitational resistance, which is sensitive to the magnitude of this gravity vector (Wolverton and Kiss 2009; Herranz and Medina 2014). It was also shown that roots kept their skewing and waving pattern in microgravity, although this was thought to be an effect of gravity. This experiment also showed that with a directional light, roots of plants grown in orbit are strongly negatively phototropic and grow in the opposite direction to the shoot, as is observed on Earth (Paul, Amalfitano and Ferl 2012).

2.4 Plants gown and used in space exploration

The concept of growing plants in space is all about developing the human technological capacity to provide plants with adequate growth conditions in the unique micro-gravity environment as it is about creating a symbiotic relationship between the plants and the space travelers. Plants in space generates numerous benefits for these space travelers (Orukpe *et.,* 2021). Some of these benefits includes; improving the quality of indoor air by helping to control humidity levels, by removing and converting carbon dioxide from air into essential Oxygen which space travelers can use for breathing. Central to the concept of bio-regenerative life support system for space exploration is the use of photo-synthetic plant and light to generate oxygen and food. Growing

plants in space may also generate a psychological and neurocognitive benefits, which can help reduce stress and tension during spaceflights.

Over the years, different plants have been able to thrive and grow in space. Some of the plants that have achieved this success includes:

- *Arabidopsis* (Thale cress)
- Bok choy (Tokyo Bekana) (Chinese cabbage)
- Super dwarf wheat
- Apogey wheat
- *Brassica rapa*
- Rice
- Tulips
- *Kalanchoe*
- Flax
- Lettuce and Cinnamon basil
- Cabbage
- Zinnia hybrida ("Profusion" var.)
- Mizuna lettuce
- Red romaine lettuce ("Outredgeous" var.)
- Sunflower
- *Ceratopteris richardii*
- Onion
- Cucumber
- Peas
- Potatoe
- Maize

- Tomato

1. **Lettuce (*Lactucasativa*)**: Lettuce is a popular candidate for space farming due to its short growth cycle, small space requirements, and high nutritional value. It is a leafy-green vegetable-rich in essential vitamins and minerals, including vitamin C, vitamin K, and folate. NASA and other space agencies have conducted multiple experiments on the International Space Station(ISS)to grow lettuce successfully in microgravity conditions. Notable experiments include the Veggie experiment on the ISS, where astronauts grew and consumed lettuce harvested in space. (Monje *et al.,*2003).

2. ***Arabidopsisthaliana(*Mouse-earcress)**: Arabidopsis thaliana is a small flowering plant commonly used as a model organism in plant biology research. Its small size and rapid growth cycle (around 6weeksfrom seed to seed) make it suitable for microgravity experiments. Arabidopsis has been extensively studied on the ISS and other space missions to understand the effects of microgravity on plant growth, development, and gene expression. Insights gained from studying Arabidopsis can also be applied to other crops. (Kwon and Sparks,2019).

3. **Soybean (*Glycinemax*)**: Soybean is a valuable crop due to its high protein content and versatility in various food products. It has been studied in space environments to evaluate its adaptability to microgravity and its potential as a protein-rich food source for astronauts during long-duration missions. NASA has conducted experiments with soybeans on the ISS as part of their efforts to develop sustainable space farming systems (Monje *et al.,*2003).

4. **Peanut(*Arachishypogaea*):** Peanuts have been considered for space farming due to their nutritional value, especially as a source of protein and healthy fats. They are also relatively easy to grow and can fix nitrogen in the soil, which is beneficial for space farming systems. (Monje *et al.,*2003).

5.**Maize** (***Zea mays)***: Corn has been studied for its adaptability to space environments and its potential as a staple-crop. It is a high-yielding plant and can be used for food, animal feed, and industrial purposes. (Paul, Amalfitano and Ferl 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Study Site

The experiment was conducted at the Space-Earth Environment Research Laboratory, University of Benin, under the Centre for Atmospheric Research of the National Space Research and Development agency (NASRDA).

3.1 Selected Crop collection.

The maize seeds were obtained from the seed bank of space-earth environment research laboratory, University of Benin, Edo State, Nigeria.

Figure 3: Maize seed (*Zea mays*) located at the seed bank of Space-Earth Environment Research Laboratory.

Photo credit (Alex Orukpe)

3.2 Apparatus and Equipment

The following apparatus and equipment were used in the course of this experiment. They include; a petri dish, beakers, measuring cylinder (100 and 1000ml), forceps, spatula, a microscope, 2D clinostat, a weighing balance, a stopwatch and a heater.

3.3 Materials and reagents

The following materials were used in the course of the experiment been carried out. They Include maize seeds (specifically selected for this study due to its small seeds, making it easy to handle, and having a 3-5day germination period and thus making it useable on the clinostat), Agar Agar, and growth stimulants;100ppm and 500ppm of

Indole acetic acid (IAA), 100ppm and 500ppm Sodium nitroprusside (SNP), tape, cotton wool, crystal violet.

3.4 Exposure of seed to water

Into a clean beaker, 20ml of distilled was poured. 40 maize seeds were placed into the beaker and were soaked for a period of one hour. After the desired time was attained and decantation had been carried out, the maize seeds were placed on a sterile filter paper to air dry, according to a relevant method (Orukpe *et al.,*2021).

3.5 Exposure of seeds to growth stimulators

3.5.1 Preparation of 100ppm of IAA (INDOLE ACAETIC ACID)

Into a clean beaker, 250ml of water was poured and then 0.025g of IAA was weighed and placed into the beaker and was stirred continuously. The beaker is stirred continuously until it is dissolved completely.

3.5.2 Preparation of 500ppm of IAA

Into a clean beaker, 250ml of distilled water was poured. 0.125g of IAA was weighed on the weighing balance and placed into the beaker, which was the stirred continuously to dissolve completely.

3.5.3 Preparation of 100ppm of SNP (Sodium nitroprusside)

0.025g was weighed and dissolved in 250ml distilled water and stirred continuously until it was properly dissolved.

3.5.4 Preparation of 500ppm of SNP

0.125g was weighed and into a beaker containing 250ml of distilled water, poured. After the preparation of the growth stimulators, the maize seeds is then subjected to the growth stimulators for a period of one hour and which decanting and then placing on a clean filter paper and is allowed to air dry.

3.6 Agar preparation

Agar was prepared following standard methods according to Orukpe *et al.,* (2021). It involved dissolving 1.5g and 0.74g of agar agar in 100ml and 50ml of distilled water respectively. Shake properly to completely dissolve and then place on a heater for about 5-10 minutes to dissolve completely while stirring. After it has completely dissolve, pour into two clean petri dishes at equal volume, and then allow to cool and solidify a bit before sowing.

3.7 Sowing the Maize seed

The maize seeds were weighed individually using a digital weighing balance, with model NO.NBT-A200; with an average of each seed to be 0.29g. The seeds were then inserted into the petri dishes containing the cooled agar. A total of 20 seeds were sown on each of the petri dishes, based on their carrying capacity. After inserting the seeds, they were then sealed properly using a masking tape. The petri dish containing the control was left under the influence of gravity on a balanced table, while the petri dishes meant for a stimulated micro-gravity environment was placed on a clinostat for 120hrs at a rotation level of 0.5rpm (revolution per minute) and 2.5rpm.

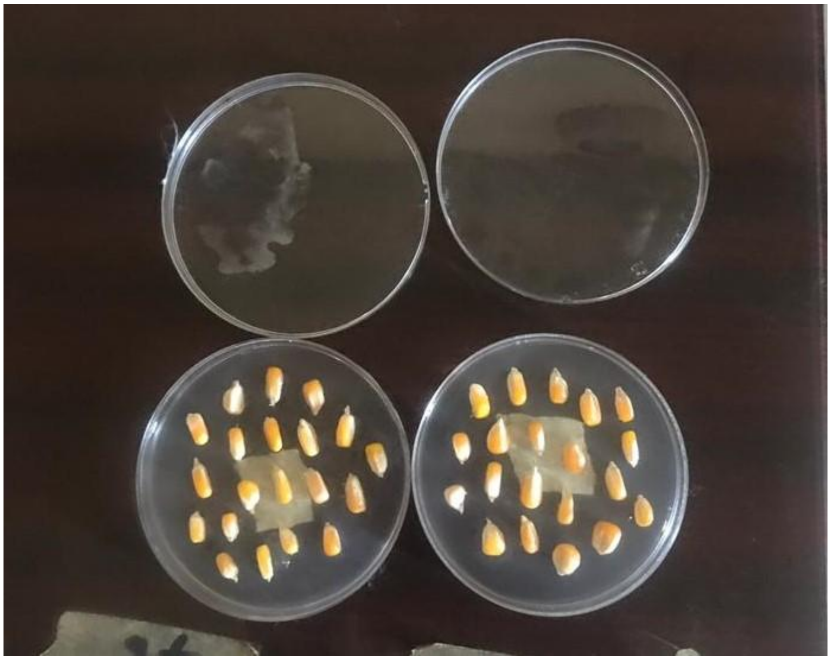


Plate 1: Maize seeds sown into the cooled agar

Photo credit (Orukpe *et al.,* 2021)

3.8 Subjecting to Clinorotation

The experiment was prepared in several batches with the different growth stimulators and at different revolution per minutes (0.5 and 2.5rpm) and was observed within 120hours, while the control containing the maize under the influence of gravity, were placed on the laboratory benches for the same duration and observation was taken every 24hours, to ascertain the differences.

Plate 2: Maize seeds grown in a stimulated micro-gravity environment using a clinostat and maize seed grown under the influence of gravity.

Photo credit (Orukpe *et al.*)

CHAPTER FOUR

RESULTS

Table 4.1: Yield parameters of maize plant exposed to microgravity

Treatment	Ear weight (g DW)	Cob weight (g DW)	No. of seeds per cob	Shelling (%)	Seed wt. per cob (g DW)
Control (G) Water	92.5	73.5	104	79.4	59.4
Water (μg) 0.5rpm	77.2	68.6	112	88.9	49.7

Water (µg) 2.5rpm	95.2	74.2	137	77.9	57.7
IAA 100 Ctr(G)	95.3	76.3	189*	80.1	54.9
IAA 500 Ctr(G)	107.4*	78.6	204*	73.2	53.9
IAA 100(µg) 0.5rpm	115.1*	81.6	124	70.9	58.5
IAA 100(µg) 2.5rpm	91.6	77.5	194*	84.6	54.8
IAA 500(µg) 0.5rpm	99.8	89.3*	160*	89.5	42.3*
IAA 500(µg) 2.5rpm	63.5*	46.7*	150*	73.5	38.9*
SNP 100 Ctr(G)	82.9	68.7	95	82.9	43.8
SNP 500 Ctr*(G)*	61.8*	46.3*	118	74.4	37.5*
SNP 100(µg) 0.5rpm	113.8*	90.7	102	79.7	61.4
SNP 100(µg) 2.5rpm	147.3*	123.5*	302*	83.8	98.5*
SNP 500(µg) 0.5rpm	140.7*	118.3*	324*	84.1	85.7*
SNP 500(µg) 2.5rpm	134.6*	105.2*	284*	78.2	86.7*
LSD (0.05)	19.4	13.2	41	15.25	18.4
p-value	<0.001	0.027	0.003	0.263	0.015

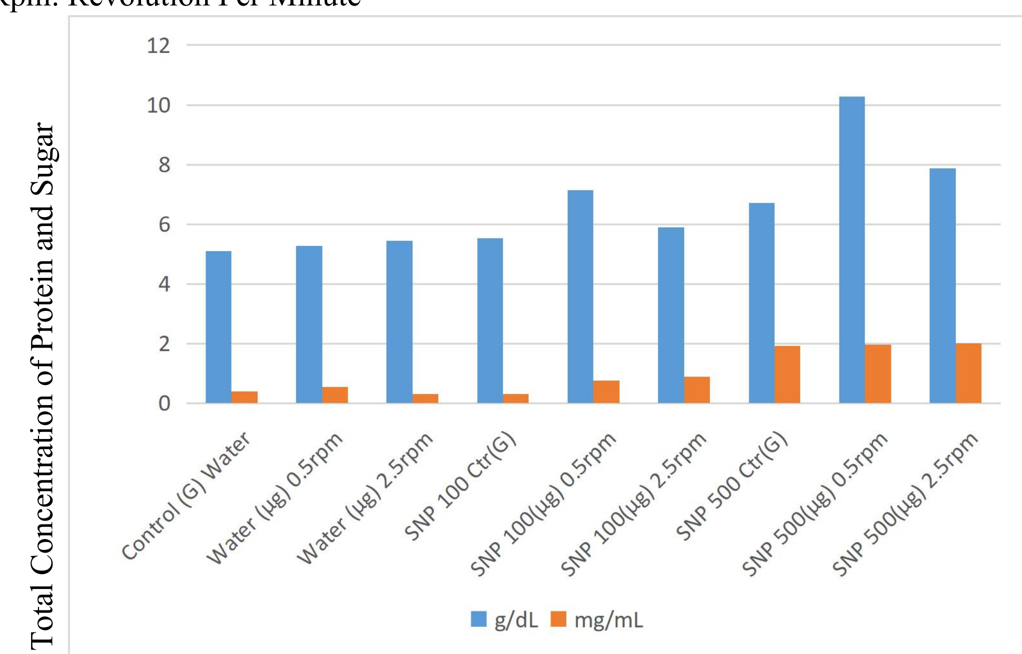
**means on similar column with asterisks differ significantly from the control (p<0.05); rpm - revolutions per minute in the 2D clinostat; SNP sodium nitroprusside, IAA Indole acetic acid; G under normal gravity; LSD Least significant difference.*

Table 4.2: Concentration of total sugar and total protein of maize seeds in microgravity and gravity environment

SAMPLE ID	PROTEIN g/dl	TOTAL SUGAR mg/Ml
Control (G) Water	5.094923	0.390719
Water (µg) 0.5rpm	5.273692	0.540419
Water (µg) 2.5rpm	5.452462	0.315868
SNP 100 Ctrl(G)	5.541846	0.311377
SNP 100(µg) 0.5rpm	7.150769	0.757485

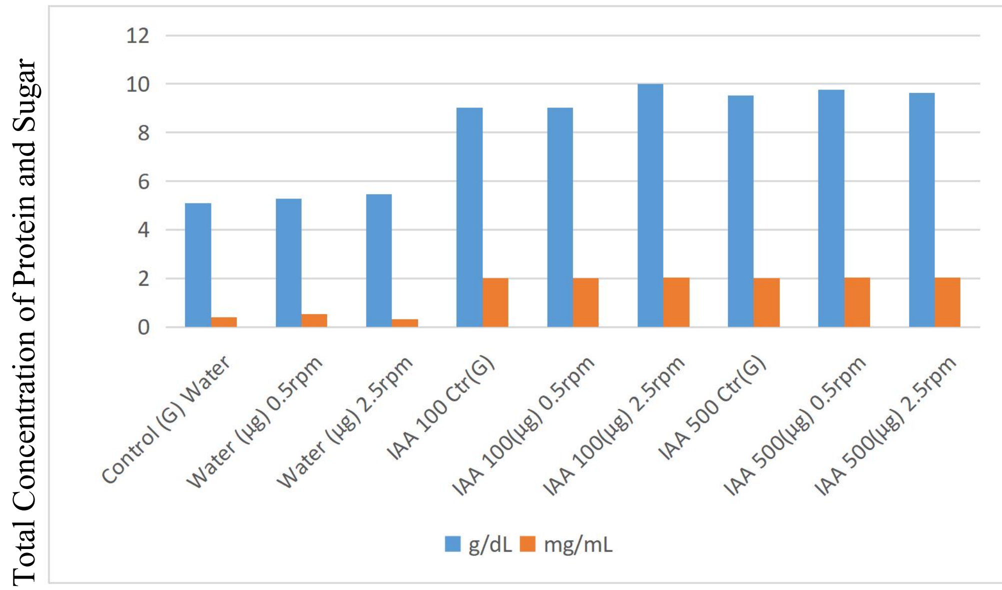
SNP 100(µg) 2.5rpm	5.899385	0.881737
SNP 500 Ctrl(G)	6.703846	1.926647
SNP 500(µg) 0.5rpm	10.27923	1.971557
SNP 500(µg) 2.5rpm	7.865846	2.004491
IAA 100 Ctr(G)	9.027846	2.004491
IAA 100(µg) 0.5rpm	9.027846	2.008982
IAA 100(µg) 2.5rpm	10.01108	2.035928
IAA 500 Ctr(G)	9.519463	2.022455
IAA 500(µg) 0.5rpm	9.765272	2.029192

KEY:
Ctrl: Water
SNP: Sodium nitroprusside
IAA: Indole-3-Acetic Acid
µg: Micro-gravity
g: Gravity
PPM: Parts Per Million
Rpm: Revolution Per Minute



Concentration of Growth Stimulant at Different Revolution per minutes

Figure 4a: Bar-chart representation of the concentration of total protein and total sugar.



Concentration of Growth Stimulant at Different Revolution per minutes

Figure 4b: Bar-chart representation of the concentration of total protein and total sugar.

KEY: SNP: Sodium nitroprusside, IAA: Indole-3-Acetic Acid, µg: Micro-gravity, g: Gravity, PPM: Parts Per Million, Rpm: Revolution Per Minute, g/dl: gram per deciliter, mg/ml: milligram per milliliter.

Germination Properties

Several plant germination parameters were measured, including the amount of time, the first appearance of germination, root length, shoot length, number of prominent roots and weight of seeds after germination. All parameters were calculated according to relevant protocols (AOSA,1983)

Plate 3: Germination of maize seed after clinorotation

Photo credit (Alex Orukpe)

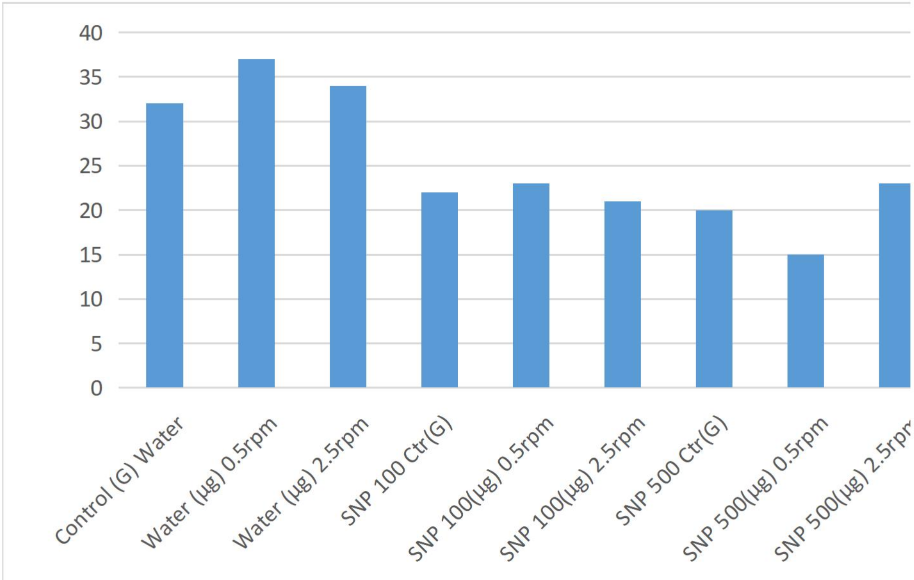


Figure 5a: Bar-chart representation of first germination

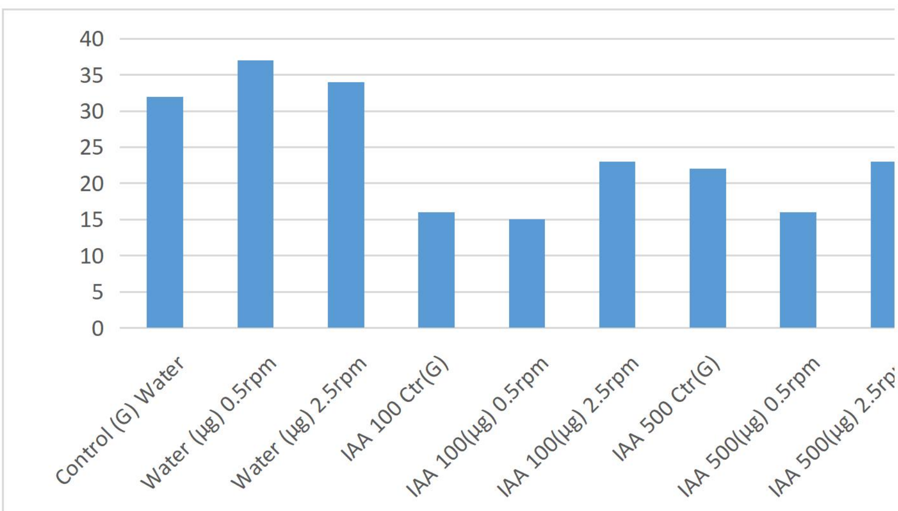


Figure 5b: Bar-chart representation of first germination

KEY: SNP: Sodium nitroprusside, IAA: Indole-3-Acetic Acid, μg: Micro-gravity, g: Gravity, PPM: Parts Per Million, Rpm: Revolution Per Minute.

CHAPTER 5

DISCUSSION

A total of 40 maize seeds were collected from the seed bank of space-earth environment research laboratory, University of Benin, Edo State, Nigeria on which analysis was done on them. The findings shown in Table 4.1 indicates the Yield parameters of maize plant exposed to microgravity which were exposed to different growth stimulants. From the table, it shows that treating the maize seeds with different stimulants such as sodium nitroprusside (SNP) and indole acetic acid (IAA) at different concentration and under different revolution per minute gave some distinct results between seeds grown in a stimulated micro-gravity environment (μg) and those grown under gravity (g). After maturation of this maize seed on the field, it showed that at different concentration of the growth stimulant applied to the seed, significant differences were observed. The Ear weight, cob weight, number of seeds per cobs, shelling and the seed weight per cob were all taken into consideration. From Table 4.1 it explained that at different treatment, the parameters differ as it progresses. Using water as a control under microgravity it showed that the ear weight measured 92.5g, whereas at a 2.5rpm (revolution per minute) using the same control, the ear weight of the maize plant measured 95.2g showing a higher difference when this maize seed was subjected to a stimulated environment using a 2D CLINOSTAT. For those treated with IAA and SNP at a concentration of 100 and 500 ppm (part per million) respectively it was observed that the maize ear weight value was higher under those grown in a stimulated microgravity environment depending on the concentration used and the revolution per minute (0.5 and 2.5 respectively) using the 2D CLINOSTAT compared to those under control. For the cob weight, significant differences were observed for the maize plant under gravity (control) and those under a stimulated microgravity. Cob weight were higher in the stimulated microgravity, and this was based on the concentration of stimulant used for treatment and at different rotation grown (0.5 and 2.5rpm respectively). The number of seeds per cob were observed to be more for those maize plants grown under stimulated microgravity compared to those under control after counting was done. It was observed that the seeds of the maize plant under microgravity were all in good conditions compared to those under control. The seed for each cob were weighed as seen in Table 4.1 and from the table it shows that for seed grown under control, they had varying weights compared to those grown under stimulated microgravity, were all seeds treated with different concentration of stimulant and grown at different grown in different revolution had seed weight that were higher.

From Table 4.2 it shows the Concentration of total sugar and total protein of maize seeds in microgravity and gravity environment after the maturation of the maize plant. From the table it shows that concentration of the total sugar and protein was influenced by the concentration of stimulant used to enhance the maize plant and the different revolution time. The total protein and sugar were higher in maize plant grown under stimulated microgravity environment compared to those grown under control. From the table it shows that the total protein 10.27923g/dl was seen to be high when treated with 500ppm of SNP (sodium nitroprusside) and at a revolution time of 0.5rpm (revolution per minutes). From the table it shows that as the concentration is increased so those the protein content increases, also depending on the revolution time been used in the stimulated microgravity environment created by a 2D CLINOSTAT. The next highest concentration of protein was seen when treated with 100ppm of IAA. It shows that at a higher revolution time of 2.5rpm, the maize plant treated with 100ppm of IAA had a protein value of 10.01108g/dl. This shows that at different concentration and revolution time, the protein value was higher in maize plants grown at a stimulated microgravity, compared to those under control.

The concentration of 100ppm of IAA at 2.5rpm shows a highest value of total sugar in the maize plant with a value of 2.035928mg/ml, with the least value of 0.311377mg/ml when treated with 100ppm of SNP under gravity. From the result on Table 4.2 it shows that the total value of protein and sugar were more in microgravity compared to those under gravity (Orukpe *et.,* 2021).

Figure 8, shows a bar chart representation of the first germination time. From the chart it was observed that seeds treated with 100ppm of SNP and IAA respectively at different revolution time will attain first germination and photosynthesis activities will begin within 15hours. This simply means that germination and photosynthesis activities will occur within this 15hours after sowing before the rest placed under gravity.

CONCLUSION

The study has revealed that maize can survive in a microgravity environment and can serve as a food source (providing good protein and sugar content), and oxygen which can be used by astronauts for long space exploration. The effect of microgravity can could impair the growth and development of the plant, but the use of stimulators like IAA (indole-3-acetic acid), SNP (sodium nitroprusside) will significantly reduce the effects of microgravity environment on *zea mays* during long space exploration and increase food security.

REFRENCES

Arena, C., V. De Micco, E. Macaeva, and R. Quintens. (2014). "Space radiation effects on plant and mammalian cells." *Acta Astronautica* **104:** 419–431.

Brown, C. S., W. C. Piastuch, and W. M. Knott. (1994). "Soybean cotyledon starch metabolism is sensitive to altered gravity conditions." *Advances in Space Research* **14** (8): 107–110.

Correll, M. J., Pyle, T. P. and Millar, K. D. (2013). Gravity effects on plant growth, development, and response to stress. Frontiers in Plant Science, **4**:494.

Crucian, B. E., Sams, C. F., Almeida, E. A., Quiriarte, H. D., Pierson, D. L. and Smith, S. M. (2018). Immune system dysregulation during spaceflight: potential countermeasures for deep space exploration missions. Frontiers in Immunology, **9**:1437.

De Micco, V., C. Arena, D. Pignalosa, and M. Durante. (2011). "Effects of sparsely and densely ionizing radiation on plants." *Radiation and Environmental Biophysics* **50**: 1–19.

De Micco, V., S. De Pascale, R. Paradiso, and G. Aronne. (2014). "Microgravity effects on plant life cycle and completion of the seed-to-seed cycle." *Plant Biology* **16** (1): 31–38.

Ferl, R., R. Wheeler, H. G. Levine, and A. L. Paul. (2002). "Plants in space." *Current Opinions in Plant Biology* **5** (3): 528–563.

Ferl, R. J. and Paul, A. L. (2016). Plants in space. Current Opinion in Plant Biology, **30**: 17-22.

Herranz, R., and F. J. Medina. (2014). "Cell proliferation and plant development under novel altered gravity environments." *Plant Biology* **16** (1): 23–30.

Hoson, T., Kamisaka, S. and Masuda, Y. (2014). Changes in plant growth processes under microgravity conditions. Pp. 31-45.

Hughson, R. L., Robertson, A. D. and Arbeille, P. (2016). Cardiovascular adaptation to microgravity: a review of research. npj Microgravity, **2**(1), 1-12.

Kering M., Zhang B. (2015). Effect of priming and seed size on germination and Emergence of Six Food-Type Soybean Varieties. International Journal of Agronomy **12**:6-16.

Kiss, J., Wolverton, C., Wyatt, S., Hasenstein,K.H., Van Loon J. (2019). Comparison of microgravity analogs to spaceflight in studies of plant growth and development. Frontiers of Plant Science **3**:12- 22.

Kittang Jost, A.-I., T. Hoson, and T.-H. Iversen. (2015). "The Utilization of Plant Facilities on the ISS the composition, growth, and development of plant cell walls under microgravity conditions." *Plants* **5**: 44–62.

Kornilova, L. N. and Kozlovskaya, I. B. (2003). Neurosensory Mechanisms of Space Adaptation Syndrome. *Human Physiology* **29**:527–538.

Kuang, A., Y. Xiao, and M. E. Musgrave. (1996). "Cytochemical localization of reserves during seed development in *Arabidopsis thaliana* under spaceflight conditions." *Annals Of Botany* **78**: 343–351.

Kwon, T., Sparks, J. A., Nakashima, J., Allen, S. N., Tang, Y., and Blancaflor, E. B. (2019). Transcriptional response of *Arabidopsis s*eedlings during spaceflight reveals peroxidase and cell wall remodeling genes associated with root hair development. American Journal of Botany, **106**(5):674-686.

LeBlanc, A., Schneider, V., Shackelford, L., West, S., Oganov, V., Bakulin, A. and Spector, E. (2000). Bone mineral and lean tissue loss after long duration space flight. Journal of Musculoskeletal and Neuronal Interactions, **1**(2):157-160.

Manzano, A.I., I. Matia, F. Gonzalez-Camacho, E. Carnero-Diaz, J.J.W.A. Van Loon, C. Dijkstra, O. Larkin, et al., (2009). "Germination of *Arabidopsis* seed in Space and in simulated microgravity: alterations in root cell growth and proliferation." *Microgravity Science and Technology* **21**: 293–297.

Massa, G. D., J. C. Emmerich, R. C. Morrow, C. M. Bourget, and C. A. Mitchell. (2006). “Plant-growth lighting for space life support: a review.” *Gravitational and Space Biology* 19 **(2)**: 19–30.

Medina, F. J., R. Herranz, C. Arena, G. Aronne, and V. De Micco. (2015). “Chapter 24: Growing plants under generated extra-terrestrial environments: effects of altered gravity and radiation.” In *Generation and Applications of Extra-Terrestrial Environments on Earth*,**24**: 239–254.

Millar, K. D., P. Kumar, M. J. Correll, J. L. Mullen, R. P. Hangarter, R. E. Edelmann, and J. Z. Kiss. (2010). “A novel phototropic response to red light is revealed in microgravity.” *New Phytologist* **186** (3): 648–656.

Monje, O., G. Stutte, and D. Chapman. (2005). “Microgravity does not alter plant stand gas exchange of wheat at moderate light levels and saturating CO_2 concentration.” *Planta* **222** (2): 336–345.

Monje, O., G. W. Stutte, G. D. Goins, D. M. Porterfield, and G. E. Bingham. (2003). “Farming in space: environmental and biophysical concerns.” *Advances in Space Research* **31** (1): 151–167.

Morrow, R. C., Remiker, R. W., Mischnick, M. J., Tuominen, L. K., Lee, M. C. and Crabb, T. M. (2005). A low equivalent system mass plant growth unit for space exploration, 35^{th} International conference on Environmental systems. Rome, Italy. Pp. 56-64

Musgrave, M. E. (2002). Seeds in Space. Seed Science Research, **12:** 1-16.

Musgrave, M. E., A. Kuang, L. K. Tuominen, L. H. Levine, and R. C. Morrow. (2005). "Seed storage reserves and glucosinolates in Brassica rapa L. grown on the International Space Station." *Journal of the American Society for Horticultural Science* **130** (6): 848–856.

Musgrave, M. E., A. Kuang, and S. W. Matthews. (1997). "Plant reproduction during spaceflight: importance of the gaseous environment." *Planta* **203**: 177–184.

Orukpe, A.O., Anoliefo, G.O., Ikhajiagbe B. (2021). Effects of Clinorotation on the Enzyme Activities and Morphology of Zea mays Seedlings. American Journal of Life Sciences **9** (1):11-18.

Papaseit, C., Pochon, N., Tabony J. (2000). Microtubule self-organization is gravity-dependent.

Paradiso, R., V. DeMicco, R. Buonomo, G. Aronne, G. Barbieri,and S. DePascale. (2014). "Soilless cultivation of soybean for Bioregenerative Life Support Systems (BLSSs): a literature review and the experience of the MELiSSA Project – Food- characteristics Phase I." *Plant Biology* **16**: 69–78.

Paul, A. L., C. L. Amalfitano, and R. J. Ferl. (2012). "Plant growth strategies are remodeled by spaceflight." *Plant Biology* **2012**: 12–232.

Paul, A. L., R. M. Wheeler, H. G. Levine, and R. J. Ferl. (2013). "Fundamental plant biology enabled by the Space Shuttle." *American Journal of Botany* **100** (1): 226–234.

Soga, K., Wakabayashi, K., Kamisaka S. (2002). Stimulation of elongation growth and xyloglucan breakdown in Arabidopsis hypocotyls under microgravity conditions in space. Planta **215**:1040–1046.

Stutte, G., Monje, O., Goins, G., Ruffe G. (2002). Evapotranspiration and photosynthesis characteristics of two wheat cultivars grown the biomass production system. SAE Technical Paper 2001-0202180.

Tuominen, L. K., L. H. Levine, and M. E. Musgrave. (2009). "Plant secondary metabolism in altered gravity." *Methods in Molecular Biology* **547**: 373–386.

Vandenbrink, J. P., J. Z. Kiss, R. Herranz, and F. J. Medina. (2014). "Light and gravity signals synergize in modulating plant." Frontiers in Plant Science **5** (563).

Vandenbrink, J. P., and J. Z. Kiss. (2016). "Space, the final frontier: a critical review of recent experiments performed in microgravity." *Plant Science* **243**: 115–119.

Wolff, S. A., L. H. Coelho, I. Karoliussen, and A.I. Kittang Jost. (2014). "Effects of the extraterrestrial environment on plants: recommendations for future space experiments for the MELiSSA Higher Plant Compartment." *Life* **4** (2): 189–204.

Wolverton, C., and J. Z. Kiss. (2009). "An Update on Plant Space Biology." *Gravitational and Space Biology* **22** (2): 13–20.